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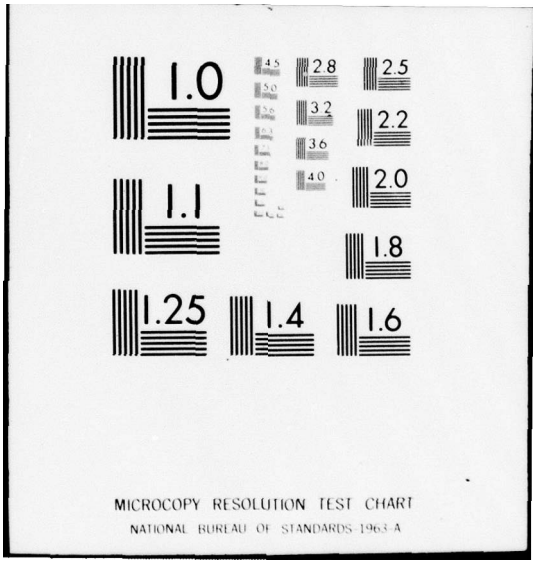
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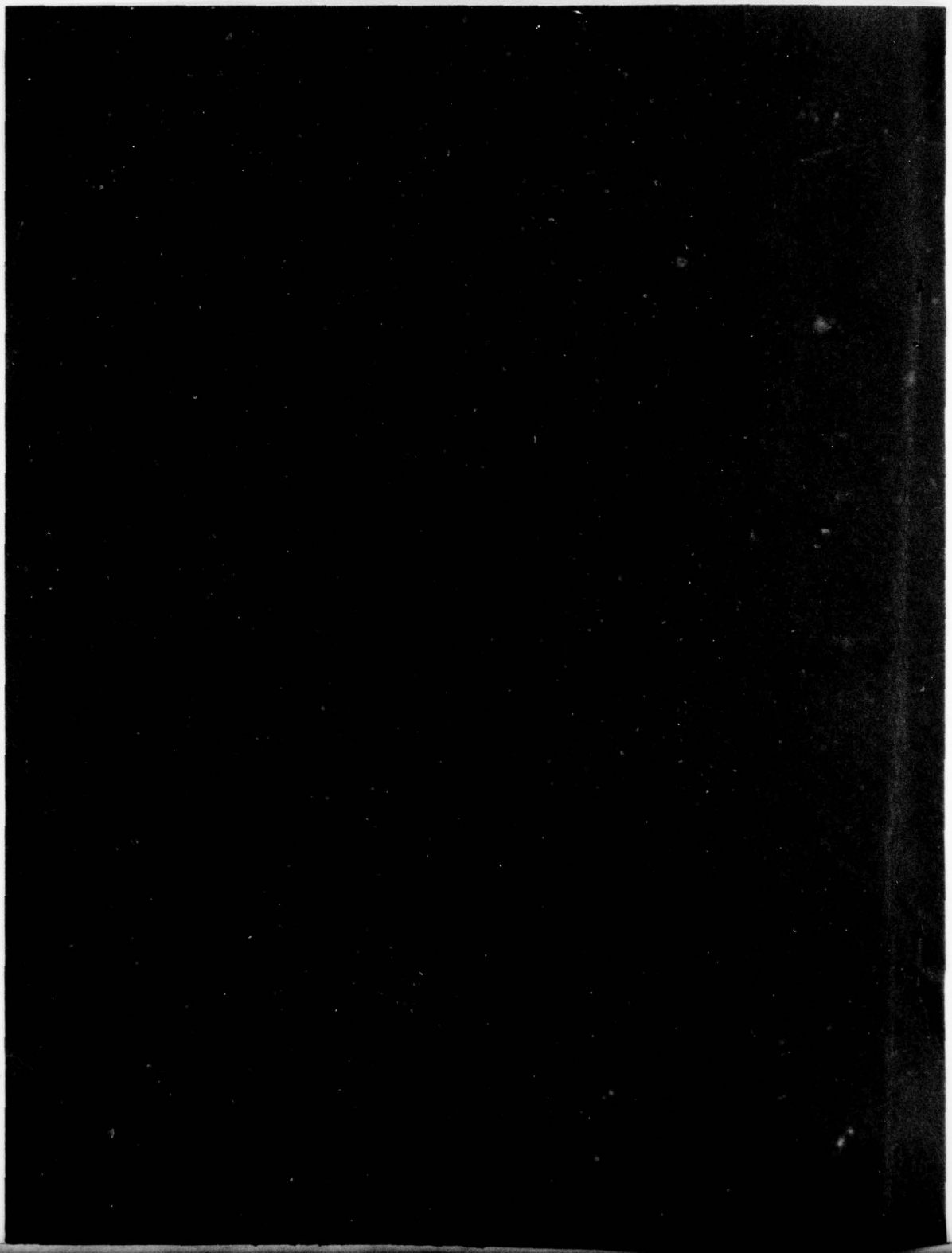

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This study was conducted to determine what effects the chronic ingestion of DDT would have on the ultrastructure of the eggshell gland of the mallard duck. The ultrastructure changes seen were edema of the type II epithelial cells as demonstrated by decreased electron density and vacuoles in the endoplasmic reticulum. Since the type II cells are responsible for the transport of calcium, alterations in these cells indicate that decreased calcium transport may be responsible for DDT-induced eggshell thinning.

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PREFACE

The work described in this report was authorized under Project/Task No. EPA. This work was started in June 1975 and completed in August 1976.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

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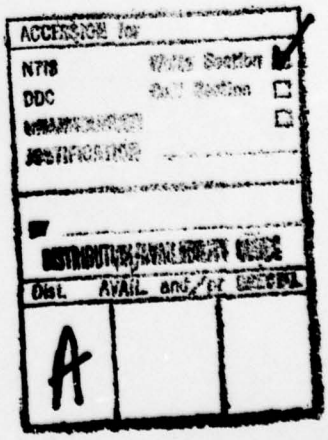

CONTENTS

LIST OF FIGURES

ULTRASTRUCTURAL ALTERATIONS IN THE EGGSHELL GLAND EPITHELIUM OF THE MALLARD DUCK AFTER CHRONIC EXPOSURE TO DDT

I. INTRODUCTION.

In 1976, Kolaja and Hinton[1] of this laboratory reported their findings on the alterations at the light microscopic level in the eggshell gland of ducks exposed to 2,2-bis-(p-chlorophenyl)-1,1,1-trichloroethane (DDT). These alterations, which accompanied eggshell thinning, included edema of the submucosa and vacuolation of the lining epithelium. Aitken (1971)[2] studied the eggshell gland from the domestic hen and described two types of epithelial lining cells: type I which had cilia on the apical surface, and type II which had both microvilli and cilia on the apical surface. The type II cells are thought to be responsible for calcium transport (Aitken, 1971).[2] The present study was undertaken to more fully characterize the alterations in the eggshell gland brought about by ingestion of DDT. For this purpose, a comparison was made between the epithelium of eggshell glands from control ducks and from ducks fed DDT; these glands were in similar stages of development.*

II. MATERIALS AND METHODS.

Mature mallard ducks *(Anas platyrhynchos)* were randomly assigned to two cages, five hens and one drake per cage. These birds were held in the cages and fed poultry-reproductive mash (Purina Chow) *ad libitum* for 1 month while they became acclimatized. One group was then fed a diet of the same poultry-reproductive mash to which had been added 50 ppm of DDT; the other group was fed the normal diet. After 5 month's of feeding, egg production was induced by regulating the photoperiod. Since the eggshell gland undergoes dramatic changes during egg production, an effort was made to compare eggshell glands that were in the same stages of development and eggs located in the same position within the tract. The following criteria are used for evaluating similarities in eggshell glands: size, degree of vascularity, and position of egg within the reproductive tract. After the birds were in full-egg production for 1 month, they were killed by cervical dislocation. The eggshell glands were rapidly excised and placed in ice-cold 4% formaldehyde, 1% glutaraldehyde in 0.1 M phosphate buffer (McDowell and Trump, 1976),[3] and stored at room temperature until time of subsequent processing. With a clean razor blade, a 0.5-mm thickness of the exterior surface of the tissue was removed and minced into pieces of approximately 0.5- by 1.0-mm in greatest dimension. The tissue was postfixed in 1% phosphate-buffered osmium tetroxide, dehydrated in graded alcohol solutions, cleared in propylene oxide, and embedded in Epon (Luft, 1961).[4] The general procedures of this study followed those methods detailed by Hayat (1970).[5] For correlation between light and electron microscopy, 1μ semi-thin tissue sections were cut and stained with toluidine blue (Trump *et al.*, 1961).[6] Thin tissue sections, 600 to 900 angstroms thick, were cut with a diamond knife and stained with uranyl magnesium acetate and lead citrate (Frasca and Parks, 1965);[7] they were then viewed with an AEI 6B electron microscope.

*In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The views of the authors do not purport to reflect the position of the Department of Defense.

III. RESULTS.

Figure 1 shows control sections of Epon-embedded material that were stained with toluidine blue. In these preparations, the nuclei of lining epithelium appeared to be stratified. However, individual cell borders could be traced from the brush border, at the luminal surface, to the basal lamina. Since the lining epithelium was one cell in thickness and the cells were columnar in shape, the tissue was classified as pseudostratified ciliated columnar epithelium. This finding is in agreement with Aitken's[2] description of the domestic hen. Nuclei of lining epithelial cells contained one to two prominent nucleoli. The cytoplasm contained granules which were dispersed from the basal to apical regions. The lining epithelium rested on a basement membrane, beneath which an abundant capillary bed and submucosal gland were seen (figure 1).

Figure 2 shows a typical semi-thin section of eggshell gland stained with toluidine blue; this was taken from a duck that was fed DDT. The major alteration encountered was swelling of the surface epithelial cells. In contrast to controls, lining epithelial cells from treated ducks revealed intracellular swelling. Cytoplasm of lining epithelium was less dense, and cell borders were more distinct than those of similar cells in control sections which revealed considerable overlapping.

Figure 3 shows the ultrastructural appearance of the epithelium of the eggshell gland from control ducks. The apical (luminal) surface had two membrane specializations: cilia and microvilli. One cell type possessed only microvilli. The other had both microvilli and cilia. For the purpose of subsequent description, nonciliated cells will be referred to as type 1, and cells with cilia will be referred to as type II. Cilia originated from well-developed basal bodies with rootlets (figure 4 arrows) extending from the basal bodies into the cytoplasm. Microtubules extended from basal bodies into cilia (see insert in figure 3). The lateral cell membranes revealed well-developed junctional complexes. Tight junctions were shown at the luminal surface; intermediate junctions extended to the cell base; and desmosomes were interspersed at intervals along the lateral surface. Undulating lateral cell membranes extended from the cell apex to the base. The cytoplasm of lining epithelium contained rough and smooth endoplasmic reticulum, secretion granules, mitochondria, and microfilaments. Secretion granules appeared as rounded structures of medium electron density. Often a single membrane surrounded individual granules. This membrane was similar in thickness to the outer mitochondrial membrane. Two morphologic regions in the matrix of secretion granules were observed. The first of these appeared as smudged areas with little recognizable structure. The second was easily recognized as a granular region. Within individual cells, secretion granules varied in appearance. Within different cell types, varying numbers of granules were encountered; type II cells contained the majority. The mitochondria were uniform in size, had numerous rows of cristae, and contained no matrical granules. Microfilaments, in large aggregates, occupied apical portions of the cytoplasm of type II cells.

Figures 4 and 5 show the typical features of ultrastructure in the epithelium of eggshell gland from a duck which had received 50 ppm of DDT for 6 months. When the epithelium of eggshell glands from DDT-treated ducks was examined using the electron microscope, a pattern of alterations which substantiated those observed in the sections stained with toluidine blue was encountered. The primary alteration was a swelling of the type II lining cells. In these cells, a simplification of the lateral infoldings was seen (figure 5). Instead of the

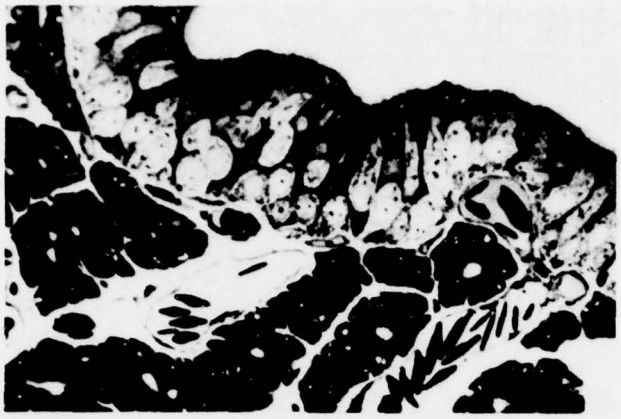

Figure 1. Eggshell Gland from Control Duck, Toluidine Blue-Stained Semi-Thin Section
Pseudostratified columnar epithelium has condensed cytoplasm. X660.

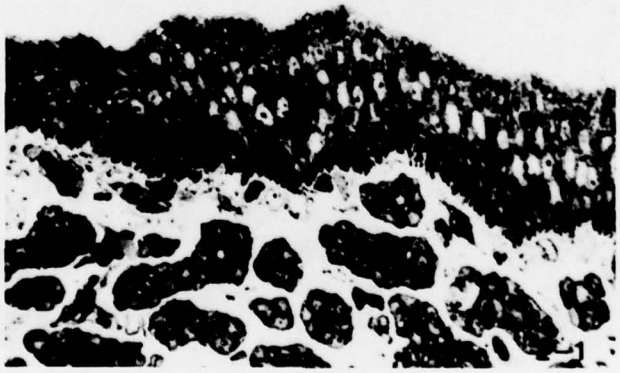

Figure 2. Eggshell Gland from Duck Fed DDT, Toluidine Blue-Stained Semi-Thin Sections
The cytoplasm of the epithelium is swollen and has more distinct cell boundaries. Type I cells have their nuclei at the basal area while type II cell nuclei are in an apical position. X660.

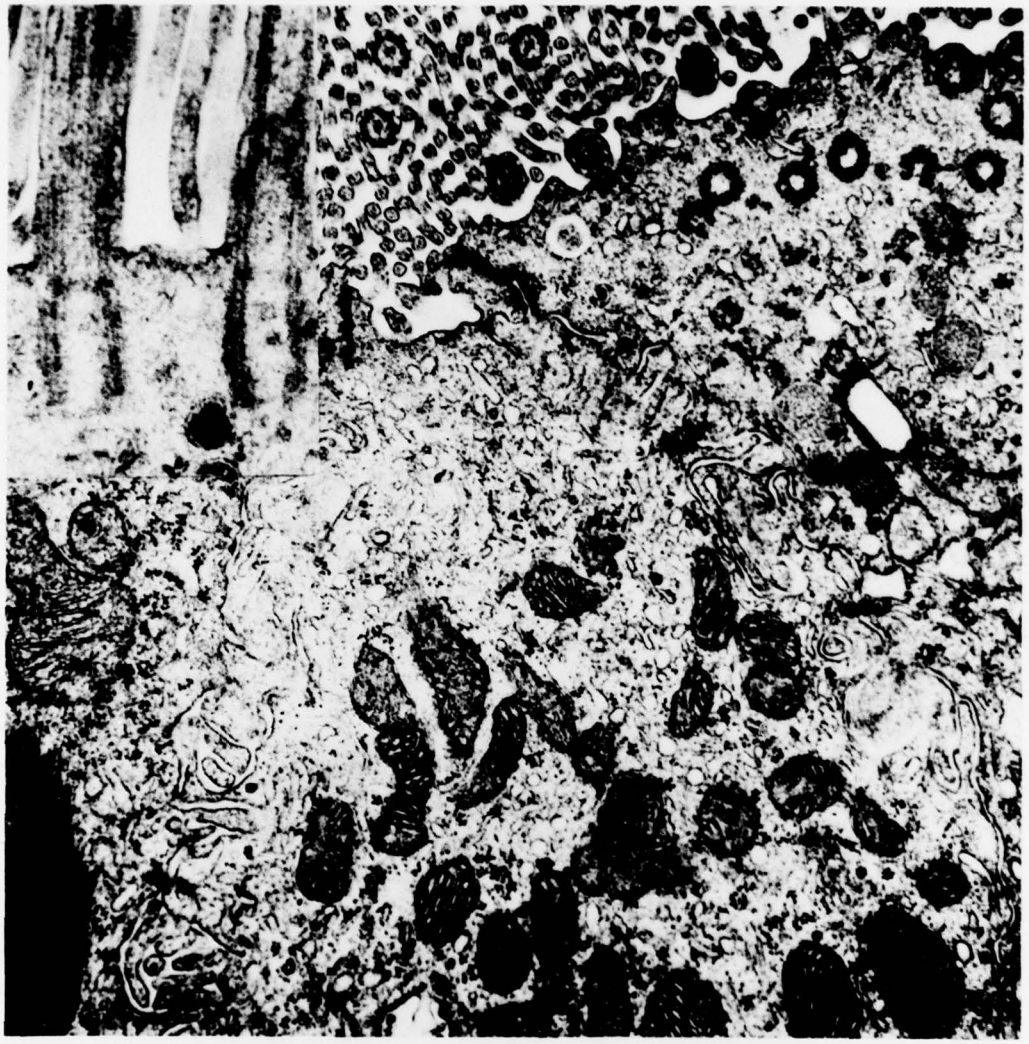

Figure 3. Ultrastructural Appearance of Control Eggshell Gland
A type I cell is in the center. The lateral cell membrane is undulating and the cytoplasm is electron dense. Note the irregular arrangement of the basal bodies. X30,000. Insert shows microtubules of cilia. X60,000.

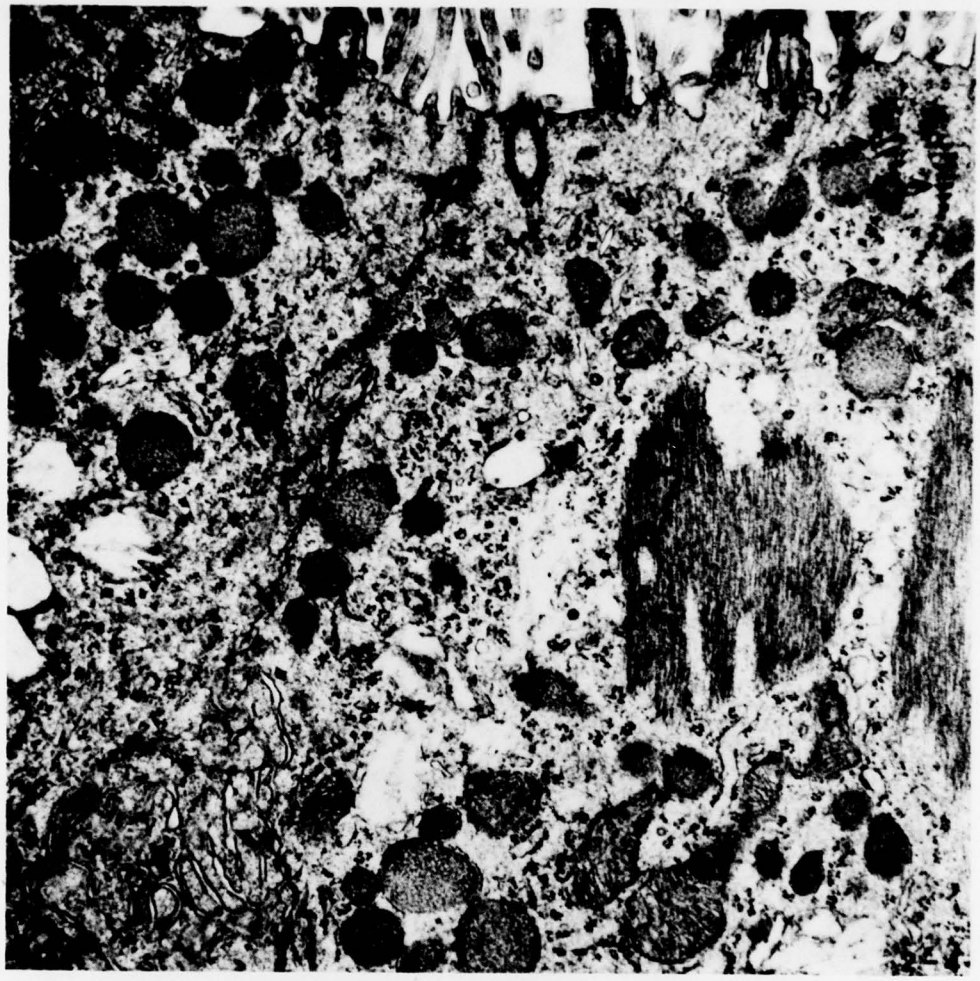

Figure 4. Eggshell Gland from Duck Fed DDT

The cytoplasm is of lesser electron density than controls; the lateral cell membrane is simplified and the cytoplasm contains vacuoles. Arrows show rootlets attached to basal bodies. X30,000.

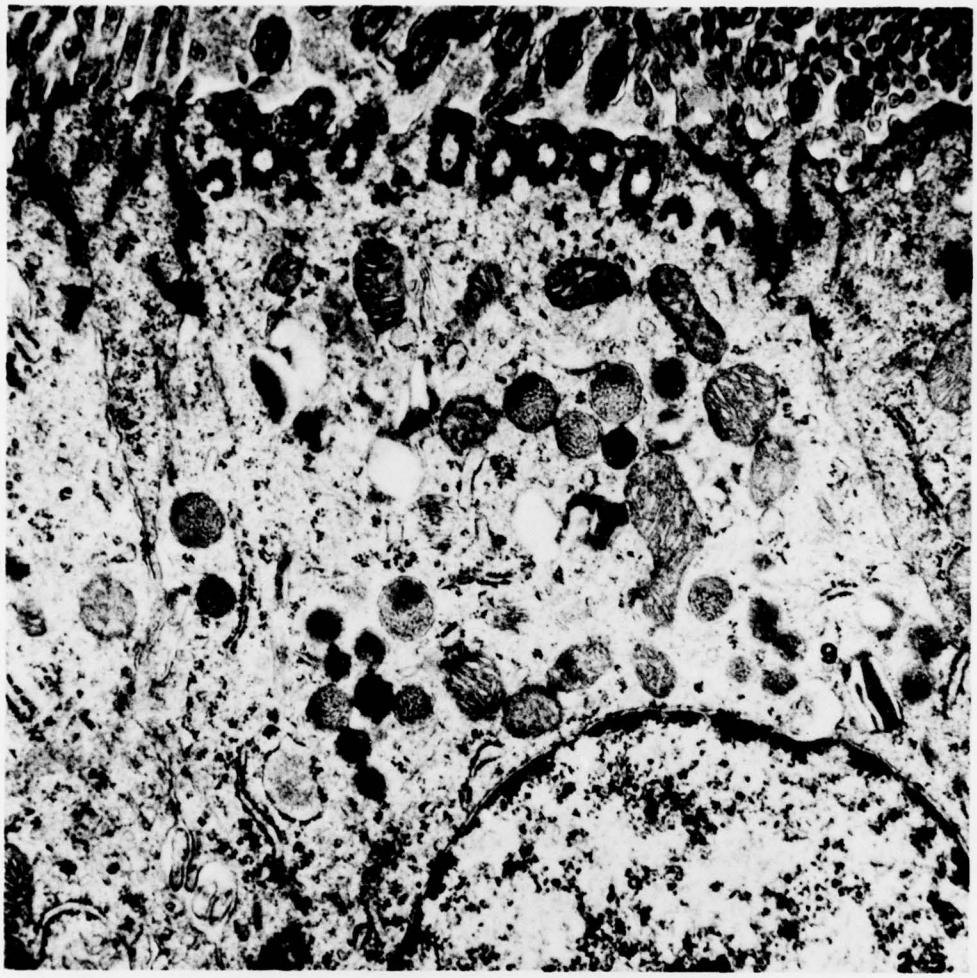

Figure 5. Eggshell Gland from Duck Fed DDT

The simplification of the lateral cell membrane and cytoplasmic vacuoles are seen in type II cells. Note the regular arrangement of the basal bodies, indicating cell swelling. X30,000.

undulating forms seen in controls, the lateral membranes appeared as straight lines. In addition, the basal bodies associated with cilia were arranged in a straight line rather than in the irregular fashion seen in controls (figure 4). Further evidence of cell swelling was seen in the cytosol which appeared to be expanded and less electron dense. The endoplasmic reticulum and nuclear envelope contained vacuoles (figures 4 and 5) not seen in controls. A flocculent material of low electron density was seen in the vacuoles.

IV. DISCUSSION.

The alterations seen at the histologic and ultrastructural level show that DDT produces morphologic changes in type II epithelial cells of the eggshell gland. Because the eggshell gland undergoes marked structural and functional changes related to the stage of egg production (Aitken, 1971)[2] and the position of the egg in the reproductive tract (Schraer and Schraer, 1970),[8] comparisons were made of eggshell glands in the same stage of development from eggs in the same position in the tract. Thus, the changes encountered in this study acquire added importance.

It is well recognized that ion shifts lead to cell swelling (Ginn *et al.*, 1968),[9] particularly in transporting epithelium such as isolated kidney tubules (Trump and Bulger, 1968)[10] and toad bladder (Saladino and Trump, 1968).[11] During DDT exposure, increasing evidence suggests that calcium transport through the gland is impaired. Miller *et al.* (1975, 1976)[12, 13] have shown decreased Ca ATPase (calcium adenosine triphosphatase) activity in mucosal preparations of Pekin ducks that were fed a DDT metabolite, 2,2-bis-(chlorophenyl)-1,1-dichloroethylene (DDE). Work in our laboratory has also shown that DDT inhibits Ca ATPase by both *in vivo* and *in vitro* administration. Impaired calcium transport could lead to an accumulation of calcium ions in one-cell compartment and cause a water influx. It is interesting to note that the defects shown in this study occurred in cells which are thought to transport calcium.

Reduced ATPase activity has been shown in other species: fish (Desaiah, 1975);[14] rat (Schneider, 1975);[15] and turtle (Witherspoon and Wells, 1975).[16] The effect of DDT on ATPase is a general effect and not limited to the bird. The inhibition of ATPase with reduced ion transport could account for ion shifts with subsequent cell swelling. The absence of more severe signs of toxicity in this study can be explained by the work of Britton (1975).[17] In his study, hens fed up to 1200 ppm of DDT for 28 days were without clinical symptoms of toxicity. The 50 ppm of DDT added to the diet in this study would be expected to produce no clinical disease in the birds.

Due to the known association of long-lived environmental pollutants such as DDT with various cell membranes and the interaction of these membranes with ion transport, it becomes increasingly apparent that correlated ultrastructural and chemical studies are needed to precisely define the mechanism of action of these potentially harmful substances.

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